activation. Here, we studied the single channel behavior of mutant sodium channels lacking fast inactivation (rat Nav1.4 L435W/L437C/A438W; Wang et al., 2003). These channels open repeatedly in response to voltage steps from −60 to 0 mV. Most of the voltage dependence of activation could be ascribed to the first latencies and the length of closures separating bursts of channel activity. Unlike non-inactivating voltage gated potassium channels which have a single open state (Hoshi et al., 1994), we observed two subconductance levels in single channels at all voltages, including during deactivation to −80, −100 and −120 mV. The dwell times in each conducting state during activation were not very voltage dependent, as were their relative probabilities following channel opening. To quantitatively explain these data, we developed a kinetic framework for the gating of these sodium channels. This work was funded by NIH grant GM084140.

3064-Plat
Use of Genetically Encoded Photoactivable Cross-Linking Molecules to Probe NaV Channel Fast Inactivation
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Fast inactivation in voltage-gated sodium channels requires efficient and rapidly reversible conformational changes in the cytoplasmic linker between domains III and IV (DIII-IV) and their putative receptor in the pore region of the channel. Introduced or acquired mutations in this complex lead to defective channel inactivation, and the resulting excessive sodium conductance can cause a myriad of pathophysiological disorders. We set out to better understand fast-inactivation by expressing voltage-gated sodium channels that carry genetically encoded orthogonal photocrosslinking (PC) amino acid molecules in the inactivation complex with the goal of capturing transient, but physiologically important complexes. PC molecules have widely been used to characterize protein-protein interactions through their ability to covalently bond with nearby molecules upon excitation by UV light. Conventional *in-vivo* use of PC molecules relies on their covalent attachment to introduced cysteine residues, which must be functionally tolerated and equally solvent accessible, and are limited by non-specific labelling of both target and off target proteins in the cellular environment. We have bypassed these limitations by utilizing the amber stop codon suppression system to genetically incorporate the PC unnatural amino acids (UAA) *p*-benzoyl-L-phenyl alanine and *p*-azido-L-phenylalanine into NaV1.5 channels in HEK cells. NaV1.5 channels carrying the UAA molecules showed robust expression at a number of sites in the DIII-IV linker with channel currents that displayed near normal activation, fast and steady state inactivation properties, verifying that the UAAs were well tolerated. Simultaneous patch clamp recording and UV irradiation of channels demonstrated that photo-activating the crosslinking UAA has effects on fast inactivation that are dependent upon the incorporation site and the state of the channel during UV exposure. This technique allows resolution of discrete, transient molecular conformations of the inactivation machinery that support sodium channel fast inactivation.

3065-Plat
Crystal Structure of a Simplified "Pore" Sodium Channel
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Voltage-gated sodium channels selectively transfer sodium across membranes in response to changes in membrane potential. The first voltage-gated sodium channel crystal structure (Payandeh et al (2011) Nature 475, 353), that of a bacterial voltage-gated sodium channel, NavAb, enabled the identification of several distinct features that differentiate these channels from the related sub-family of voltage-gated potassium channels. The NavAb structure represents a pre-open state where the voltage sensors are activated while the activation gate and pore are closed. Recently, we reported on the production and characterization of an extremely stable pore-only sodium channel from a different bacterial homologue (McCusker et al (2011) J. Biol Chem. 286:16386); it is capable of supporting Na+ ion permeation and ligand block similar to its full-length voltage-gated counterpart. We now report on the crystal structure of a sodium channel pore solved by molecular replacement using the NavAb structure. In contrast to NavAb, the pore was crystallized from detergent micelles. In the absence of the voltage sensor, the pore has a similar overall arrangement to the pore domain of NavAb but is of a more open conformation. The most prominent deviations from the pre-open state structure occur at the intracellular face of the S6 helices and in the extracellular P-loop; hence it may represent an alternate state in the process of conductance. This structure thus provides new insight into the structural basis of sodium channel ion conductance.

3066-Plat
Structural Modeling of a Human Voltage-Gated Sodium Channel
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Mammalian voltage-gated sodium channels play essential physiological roles in neuronal, cardiac, and muscle cells, but high-resolution structures of these channels remain elusive. Recently, the x-ray structure of a bacterial voltage-gated sodium channel from Arcobacter butzleri (NavAb) was solved at atomic resolution. Comparison of NavAb channel structure to Kv1.2-Kv2.1 and MlotiK1 channel structures revealed some similarities and significant differences within the voltage-sensing and pore-forming subdomains. We used the Rosetta-Membrane-Symmetry computational method with x-ray structures of NavAb, Kv1.2-Kv2.1, and MlotiK1 channels as templates to predict structure of transmembrane regions of a human voltage-gated sodium channel (hNav1.2). Structural models of hNav1.2 reveal key structural similarities and differences between each of four domains forming mammalian voltage-gated sodium channels. Unique structural features of the voltage-sensing subdomains in domains II and IV of hNav1.2 suggest possible reasons why scorpion toxins evolved to target the voltage-sensing subdomains in domains II and IV, but not in domains I and III. Docking of pore-blocking drugs to the pore-forming subdomain of hNav1.2 using the Rosetta-Dock method reveals details of potential molecular interactions between the drugs and receptor site formed by highly conserved residues in the pore lumen. High-resolution structural modeling of human voltage-gated sodium channels may be useful for computational and chemical design of novel subtype-specific therapeutics for treatment of pain, epilepsy, cardiac arrhythmias, and other diseases associated with function of voltage-gated sodium channels.

3067-Plat
Ion Binding Sites and Hydration in the Selectivity Filter of the Bacterial Sodium Channel Navab
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Molecular dynamics (MD) calculations have been used to identify Na+ binding sites in the selectivity filter of the bacterial voltage-gated sodium channel NavAb. MD trajectories, carried out starting from the recently reported X-ray crystal structure in the closed-pore conformation, revealed two locations in the selectivity filter of the channel, with Na+ coordinated to both water molecules and negatively charged protein residues. The dynamic nature of the binding of Na+ to the selectivity filter allows waters to permeate from bulk to the central cavity, without displacing the bound ions, a finding that contrasts with the situation observed for potassium channels.

3068-Plat
Monovalent Ion Selectivity of the Homotetrameric Bacterial Na Channel, NaChBac
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NaChBac, from *Bacillus halodurans*, is a homotetrameric Na_v channel, with each monomer having 6 putative transmembrane helices. Its selectivity filter appears to be lined by a ring of 4 glutamate residues (E191 from each monomer). We expressed NaChBac channels in HEK293 cells and studied their selectivity to monovalent organic and alkali cations. Reversal potential shifts were determined from whole-cell currents, following substitution of a test cation for extracellular Na^+, and relative permeabilities (P_X/P_{Na}) were calculated using the Goldman-Hodgkin-Katz equation. Reversal potentials (E_{rev}) were routinely measured from plots of peak I_{Na} vs V, but for ions showing small inward currents, values were checked against tail currents, measured immediately after a maximally activating prepulse. Among the alkali cations, only Na^+, Li^+ and K^+ were measurably permeant. Of 14 organic cations, at pH 7.4, only hydrazinium (HZ^+) was measurably permeant (P_X/P_{Na}, ≈ 0.8, 0.3, 0.7 respectively, for X= Li, K, HZ; <0.1 for Rb and ammonium). In contrast to eukaryotic, 4-domain Na_v channels, neither ammonium nor hydroxylammonium (HA^+) was measurably permeant. The NaChBac mutant, E191D, showed diminished selectivity, reflected by increased values for P_X/P_{Na}. This mutation conserves the charge in the putative selectivity filter ring, but likely enlarges the filter diameter because of the shorter aspartate side chains, thereby reducing the stringent selectivity attained by the WT channel. For E191D channels, ammonium (P_{NH4}/P_{Na} ≈ 0.4), as well as hydroxyl-ammonium and some other small organic ions, showed measurable permeability. The recently determined crystal structure for the related channel, NavAb (Payandeh et al., 2011, Nature 475:353), opens the way, for the first time, to interpret selectivity of a voltage-dependent Na channel in the context of high-resolution structural